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# Development of Cleaning Methods, Quality Control and Standardization, Comparative Study of Acute Toxicity and Specific Activity of Mumiyo

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**Abstract:**Mumiyo is a natural resinous product, which is shapeless lumps with an uneven-cellular or smooth surface, hard or elastic consistency, with a characteristic balsamic smell. Mumiyo consists of organic and inorganic parts; in the inorganic part - the minerals calcium, sodium, potassium, magnesium and aluminum. In addition, about 23 more elements are present in the inorganic part: aluminum, lead, tungsten, magnesium, zinc, silicon, iron, manganese, tin, cadmium, chromium, sodium, copper, titanium, bismuth, nickel, molybdenum, calcium, potassium, cobalt, vanadium, phosphorus, mercury. The organic part of the mumiyo contains hippuric, benzoic, fatty, humic and amino acids, as well as steroids and vitamins, including vitamin D_3. Mumiyo dissolves well in water. The pH of a freshly prepared solution ranges from 6.7 to 7.0, and during long-term storage it increases to 7.5.

Determination of the authenticity and good quality of the purified mummy was carried out by the data of infrared spectra and color reactions. A comparative study was made of the acute toxicity and wound-healing effects produced by PHARM ENGINEERING LLC “Mumiyo asil ACP” in comparison with the drug “Mumiyo asil” produced by JV REMEDY GROUP LLC. The results of pharmacological experiments showed that they are biologically equivalent to those with low toxicity in terms of acute toxicity, the drugs have a wound healing effect.

**Keywords**: mumiyo asil ACP, authenticity, acute toxicity, wound healing effect, bioequivalence, chemical composition, vitamin 〖D〗 _3, humic acids, hippuric acid, steroids, amino acids, fatty acids, solubility, standardization.

Mumiyo is a natural resinous product, which is shapeless lumps with an uneven-cellular or smooth surface, hard or elastic consistency, with a characteristic balsamic smell, after appropriate processing, which has a wide range of biological effects [1-3].

Mumiyo (Greek) - "preserving the body" or "protecting the body." Translated from the Arabic "arakuljibol" - mountain sweat, from the Tibetan and Mongolian "brag-shun" (or "brag-jun)" - the juice of a rock, from the Burmese "kao-tui" (or "chaotui") - the blood of the mountain. In Turkmenistan, mumiyo is called "mumnogay", in Uzbekistan - "mumiyo asil" is a real mumiyo, in Tajikistan - "zogh" or "kiyom", in Kyrgyzstan - "arhar-tash" (or "kiik-tash", "momiya", “Momlai”, “monuya”, “ular-tash”), in Tuve - “chukla der”, in Siberia - “barakhshin” (mountain or stone oil, mountain tar [4].

For the introduction of mumiyo into official medicine, the primary task is to develop accurate, modern methods of purification, drying and methods of quality control of the substance and standardization of mumiyo and its dosage forms.

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All the methods of purification that have existed until now boiled down to the fact that the mumiyo was dissolved in a large amount of water, then, after dissolution, it was filtered through gauze and evaporated to a thick consistency [2,3].

After the publication of A.Sh. Shakirov on the high effectiveness of mumiyo in the treatment of injuries of the musculoskeletal system, there is a need to develop precise methods of standardization for mumiyo in order to introduce it into medical practice. We were given the task to develop an innovative method of purification and quality control, as well as standardization of mumiyo, preparation of regulatory and technical documentation for it and the development of its dosage forms.
experimental part

Materials and methods. As an object of research, samples of mumiyo from various deposits were used:
Sample No. 1 - production area Samarkand region (Uzbekistan);
Sample No. 2 - production area Tashkent region (Uzbekistan);
Sample No. 3 - production area of Fergana region (Uzbekistan);
Sample No. 4 - mining area, Kadamdzhai settlement (Kyrgyzstan);
Sample No. 5 - mining area, Khaidarkan settlement (Kyrgyzstan).
All specimens are red-brown to dark brown chunks with a pungent odor of animal excrement. The active substances of mumiyo dissolve well in water, poorly in organic solvents.
Each sample of mumiyo was subjected to purification according to the technology developed by us. When developing standardization methods, 5 series of each sample were analyzed.
The research objects were:

Samples of mumiyo from various deposits.
Methods used:
-extraction;
-IR spectroscopy;
-chromatography;
-HPLC
Devices:
-IR spectra were recorded on the surf "Avatar-360" (USA).
-amino acid analysis was carried out on the amino acid analyzer "Amino-Acid Analyzer T 339", Microtechna Pragul Chehoslovakia
- fatty acid analysis was carried out on a chromatograph "Chrom-4".
-Vitamin D_3 on a Knaver chromatograph:

**Obtaining purified mumiyo (powdered substance mumiyo).**
Mumiyo-raw is crushed. 278 g of crushed rock is placed in a container, where 2.78 liters of hot water are poured. The resulting suspension (3.05) is transferred to a dissolution apparatus. The dissolution process is intensified by the imposition of an ultrasonic field at 30-35 Hz for 15 minutes. The resulting solution of mumiyo in the amount of 2.72 liters is subjected to vacuum filtration, and then ultrafiltration. The ultrafiltration process takes place at a constant temperature t = 400C. The resulting 2.52 l of filtrate is evaporated under vacuum to form 1.27 l of one stripped off solution, which is dried in a vacuum chamber created at the Tashkent State Technical University in cooperation with Remedy Group LLC.

It should be noted that the process of cleaning mumiyo requires high-speed and low-temperature drying. This mode was implemented in the developed installation. In a chamber measuring (3.8x2.2) 9 m ^ 3, a vacuum P = -0.8 atm is created. In the racks inside the chamber, a mumiyo

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with a moisture content of 16-20% is placed, subject to dehydration. When the water vacuum pump (VVH) and IR heating are turned on, 3 kW of energy (for the pump) and 10 kW (heating) are consumed at the maximum cabinet load.

We considered drying mumiyo in discrete and continuous modes. In discrete mode, the IR heating should be turned off from time to time. This mode contributes to the preservation of biologically active components and energy conservation. During the shutdown of IR heating, the power consumption is associated only with the energy consumption of the vacuum pump motor. Calculations have shown that the power gained will be 10% in one discrete cycle. But increasing the number of cycles will reduce the drying speed. In this case, as a rule, additional labor costs arise.

It should be noted that drying mumiyo in a conventional vacuum drying oven is accompanied by large losses of an expensive product, as well as partial destruction of active components. Drying time is reduced by 5-6 times. The final product obtained in the infrared vacuum unit is distinguished by its quality indicators: the appearance is brown, against black-brown, the pH of 0.1% solution is 7.8, and in conventional drying the pH is 9.1, and bioactive components are also preserved.
Thus, the selected mode of drying mumiyo in the "IR vacuum installation" improves the appearance of the final product, reduces the loss of valuable raw materials. Preservation of bioactive components and lowering the pH value of the medium of aqueous solutions [5].

**Purified mumiyo properties:**the resulting purified mummy is a dark brown (or amber-brown), rosin-like, molecularly dispersed, plastic, sticky substance, sometimes fragile. Mumiyo gives a glassy-conchlike fracture with numerous acute-angled, translucent small fragments, with a characteristic bright waxy (or resin) sheen. In polarized light at 50x magnification, acicular crystals separated by a resinous substance can be seen. The density of the purified mumiyo varies (depending on the place of extraction) from 1.8 to 2.2. It does not freeze in frost, at -200C, retaining stickiness and plasticity. It melts in the range of 80-2000C, at 150-2000C it releases water and carbon dioxide. At 350-4000C, decomposition begins and complex chemical transformations take place. Complete decomposition occurs at 600-7000C, with further heating, the mineral component is also destroyed. In an open flame, the mumiyo burns without soot, leaving an ash-gray ash. During long-term storage due to moisture loss, the mumiyo hardens [6-7].

**Solubility:**mumiyo dissolves well in water (in a ratio of 1: 8), much worse in alcohol (1: 4500) and in ether (1: 7000), poorly soluble in chloroform (1: 10000). Aqueous solutions foam, alcohol solutions - too, but to a lesser extent. When moistened, the mumiyo softens and forms a pasty mass. An aqueous solution of mumiyo has a brownish color, with a tinge of strong coffee or dark beer. This solution has a bitter taste and a strong specific smell, in which the smells of chocolate and bitumen are mixed at once - in combination.
It should be noted that the pH of a freshly prepared solution ranges from 6.7 to 7.0, and during long-term storage it increases to 7.5.

**Chemical composition**: the organic part of the mumiyo has the empirical formula $C_6H_6O_8$ and is a complex of compounds with hydroxyl, carbonyl and aldehyde groups. The empirical formula of the inorganic part is as follows: $CaSi(K, Na)_5C_{25}H_5O_{26}$.
Numerous macro- and microelements are found in mumiyo, which determine many of its medicinal properties.

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The inorganic part of the mumiyo contains chemical elements that are both in a free state and in the form of oxides and salts, and makes up about 30% of the total mass of the mumiyo. Moreover, the share of potassium, calcium and magnesium accounts for more than 20% of the total mass of the mummy.
When studying the mineral composition, 23 elements were found: aluminum, lead, tungsten, magnesium, zinc, silicon, iron, manganese, tin, cadmium, chromium, sodium, copper, titanium, bismuth, nickel, molybdenum, calcium, potassium, cobalt, vanadium , phosphorus, mercury. The determination of the mineral composition was carried out by spectral analysis.
To identify the organic part of mumiyo, a method was developed based on distillation with water vapor, as a result of which the sum of two groups of substances was obtained: hippuric acid and steroids.

The presence of hippuric acid is proved by the following reaction: with the help of a correct needle, several crystals are transferred from the resulting residue to a watch glass, 3-4 drops of diluted hydrochloric acid are added and slightly heated. After cooling, add a few drops of ammonia solution until neutral and add one drop of ferric chloride solution. This produces a very distinct flesh-colored precipitate characteristic of benzoic acid, a decay product of hippuric acid.
To identify steroids, the mass remaining after distillation is treated with 50% methanol solution and tested for steroids by the following reactions:
1. Liebermann-Burchard reaction: to 0.3 ml of the test solution add 3-4 drops of acetic acid, 0.5 ml of acetic anhydride and 2 drops of concentrated sulfuric acid, a red color appears.
2. Rosenheim reaction: add 0.5 ml of chloroform to 0.3 ml of the test solution, mix well, add 96% aqueous solution of trichloroacetic acid. The color appears, gradually changing from pink to lilac and intense blue.
3. Carré-Preuss reaction: 0.5 ml of a 28% solution of antimony chloride in chloroform is added to 0.3 ml of the test solution, a blue-violet color appears.
For the quantitative determination of steroids from 1 g of mumiyo, they are extracted with a mixture of water, concentrated hydrochloric acid and glacial acetic acid. After removing the steroids, the optical density of the lilac-colored upper layer of the solution is measured on a spectrophotometer at a wavelength of 515 nm in a cuvette with a layer thickness of 1 cm. 1 g of mumiyo should contain 0.060-0.092% steroids.

**Methods for determining the authenticity and good quality of the purified mumiyo**.
It was found that the IR spectra of aqueous solutions of mumiyo after purification are read in the range of 1610 〖cm〗 ^ (- 1) and 1420 〖cm〗 ^ (- 1) (-COOH); in the region of 1660 〖cm〗 ^ (- 1) and 1550 〖cm〗 ^ (- 1) (- 〖NH енными _2) with pronounced maxima in the region of 3400-3450 〖cm〗 ^ (- 1) (stretching vibrations of the OH group) , 2960 〖cm〗 ^ (- 1) (stretching vibrations 〖CH〗 _3 - groups in aliphatic hydrocarbons), 730 〖cm〗 ^ (- 1) (bending vibration of the OH group), 1550 〖cm〗 ^ (- 1) (deformation vibration 〖CH〗 _2 - in the group 〖-CH〗 _2- 〖CO -)〗 _. Moreover, it was found that the nature of the IR spectra is the same for all samples studied samples of mumiyo [8].
Color reactions with ferric chloride (dark green color) and diazotized sulfanilic acid (dark red color) are proposed for identification of mumiyo. It has been shown that the color of the mumiyo solution does not change when interacting with dilute alkalis, but the solution brightens and gives a brown precipitate when diluted acids are added.
Development of methods for standardization of mumiyo purified by determining the amino acid composition.

**Determination of the amino acid composition of purified mumiyo**

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About 0.05 g (accurately weighed) of the drug is placed in 20 ml heat-resistant glass ampoules and 10 ml of 6 mol / L hydrochloric acid solution is poured and sealed. The contents of the ampoule are subjected to hydrolysis for 24 hours at 1100C in an autoclave. The resulting hydrolyzate is evaporated to dryness on a rotary evaporator. 5 mg of dry residue is dissolved in 10 ml of citrate-buffer solution with pH 2.2 and 0.1 ml of the resulting solution is introduced into the analyzer of the chromatograph "Amino-Acid Analyzer T 339", Microtechna Pragul Chehoslovakia with a microsyringe. The amino acid content is calculated in nanomoles (nmol).
Amino acid analysis was performed under standard conditions commonly used for the separation of protein hydrolysates.
To calculate and interpret the obtained chromatograms, an integrator equipped with an amino acid analyzer was used.
The amino acid content was calculated in nanomoles (nmol). To determine the percentage of found free amino acids in the total amount of amino acids, we used the formula:

$$\text{Amino acid},\% = \frac{nmol \cdot M(\text{Amino acids}) \cdot 10^{-4}}{a} \times W$$

where;
$nmol$ – the amount of amino acid, nanomole;
$M$ – molecular weight of the corresponding amino acid;
$W$ – dilution, ml;
According to the data obtained, 14 amino acids are present in the analyzed sample. Of these, 5 are irreplaceable (threonine, valine, leucine, lysine, phenylalanine). The absence of sulfur-containing (cysteine, cystine and methionine) amino acids is noted. The total content of glycine and glutamic acid exceeds 40% of the sum of amino acids. The total amino acid content in purified mumiyo is 3.2-4.5%.
The determination results are shown in table 1.

**Table1.**
**Amino acid content in purified mumiyo**

| № | Amino acid | Amino acid content, % , (X) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 1 | Aspartic Acid (ASR) | 0,05 (1,36) | 0,065 (2,05) | 0,08 (1, 8) | 0,11 (2,71) | 0,12 (3,02) |
| 2 | Proline (PRO) | 0,002 (0,054) | 0,003 (0,095) | 0,001 (0,023) | 0,003 (0,074) | 0,002 (0,05) |
| 3 | *Threonine (THR) | 0,04 (1,09) | 0,05 (1,58) | 0,045 (1,01) | 0,056 (1,38) | 0,06 (1,51) |
| 4 | Serine (SER) | 0,03 (0,82) | 0,033 (1,04) | 0,046 (1,04) | 0,05 (1,23) | 0,05 (1,26) |
| 5 | Glutamic Acid (GLU) | 0,25 (6,8) | 0,148 (4,67) | 0,19 (4,28) | 0,2 (4,93) | 0,21 (5,29) |
| 6 | Glycine (GLY) | 1,8 (48,97) | 1,5 (47,29) | 2,31 (51,97) | 1,75 (43,16) | 2,05 (51,64) |
| 7 | *Valine (VAL) | 0,05 (1,36) | 0,04 (1,26) | 0,06 (1,85) | 0,08 (2,02) | 0,05 (1,37) |
| 8 | Arginine (Arg) | 1,25 (34,0) | 1,1 (34,68) | 1,48 (33,3) | 1,50 (36.99) | 1,1 (27,71) |
| 9 | Isoleucine (ILE) | 0,015 (0,41) | 0,018 (0,57) | 0,016 (0,36) | 0,022 (0,54) | 0,03 (0,76) |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 10 | * Leucine (LEU) | 0,03 (0,82) | 0,04 (1,26) | 0,02 (0,45) | 0,033 (0,81) | 0,04 (1,01) |
| 11 | Histidine (HIS) | 0,07 (1.9) | 0,065 (2,05) | 0,06 (1,35) | 0,076 (1,87) | 0,08 (2,02) |
| 12 | * Lysine (DAYS) | 0,014 (0,38) | 0,018 (0,57) | 0,016 (0,36) | 0,02 (0,49) | 0,017 (0,43) |
| 13 | Alanine (ALA) | 0,065 (1,77) | 0,08 (2,52) | 0,11 (2,48) | 0,15 (3.7) | 0,12 (3,02) |
| 14 | * Phenylalanine (PHE) | 0,011 (0,27) | 0,012 (0,38) | 0,015 (0,25) | 0,011 (0,28) | 0,011 (0,28) |
| **General content** | | 3,676 | 3,172 | 4,445 | 4,055 | 3,970 |

(X) -% content of each amino acid from the sum of amino acids.

**Determination of the fatty acid composition of purified mumiyo**

About 4 g of the drug (accurately weighed) is placed in a conical flask with a capacity of 100 ml and dissolved in 40 ml of purified water. The solution is transferred into a separatory funnel, the fatty acids are extracted by extraction with chloroform three times in portions of 10.5 and 5 ml. The combined chloroform extracts are evaporated to dryness in a porcelain dish on a water bath. To 0.0125 g of dry residue, add 10-15 ml of diazomethane in ether and leave at room temperature for methylation for 10 minutes. The resulting solution is injected with a microsyringe into the evaporator of a chromatograph "Chrom-4" or another brand with a flame ionization detector and a column 250x0.3 cm in size, filled with Reoplex-400 chromosorb.

The analysis is carried out in an isothermal operating mode of the column thermostat at a temperature of 1900C, an evaporator temperature of 2500C, a nitrogen carrier gas flow rate of 30 ml / min. Fatty acids are identified by the retention time of standard substances.

In the purified mumiyo, 12 fatty acids were determined, the total content of which is 1-4%. Table 2 shows the results of the determination.

**Table2.**
**The composition of fatty acids in purified mumiyo**

| **Acids** | Fatty acid content,% of the total fatty acids | | | | |
|---|---|---|---|---|---|
| | 1 sample | 2 sample | 3 sample | 4 sample | 5 sample |
| Caprylic | 0,0613 (2,5) | 0,0653 (2,6) | 0,0742 (2,65) | 0,10248 (3,25) | 0,1059 (3,3) |
| Capric | 0,0527 (2,15) | 0,064 (2,55) | 0,0672 (2,40) | 0,0614 (1,95) | 0,0594 (1,85) |
| Lauric | 0,0650 (2,65) | 0,0678 (2,70) | 0,0910 (3,25) | 0,1087 (3,45) | 0,8670 (2,70) |
| Myristic | 0,0649 (2,7) | 0,0690 (2,75) | 0,0854 (3,05) | 0,0898 (2,85) | 0,0835 (2,60) |
| Palmitic | 0,6880 (28,1) | 0,7078 (28,2) | 0,8344 (29,8) | 0,8300 (26,35) | 0,8844 (27,55) |
| Margarine | 0,1520 (6,20) | 0,1418 (5,65) | 0,1624 (5,80) | 0,0961 (3,05) | 0,1043 (3,25) |

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| | | | | | |
|---|---|---|---|---|---|
| Pentadecyl | 0,0502 (2,05) | 0,0490 (2,00) | 0,0563 (2,00) | 0,0642 (2,00) | 0,0496 (2,05) |
| Palmitoleic | 0,0992 (4,05) | 0,1067 (4,25) | 0,1050 (3,75) | 0,1213 (3,85) | 0,1043 (3,25) |
| Stearic | 0,1409 (5,75) | 0,1355 (5,40) | 0,1652 (5,90) | 0,315 (10,00) | 0,3017 (9,40) |
| Oleinovaya | 0,4925 (20,10) | 0,4844 (19,30) | 0,5264 (18,80) | 0,5843 (18,55) | 0,6099 (19,00) |
| Linoleic | 0,5047 (20,6) | 0,5334 (21,25) | 0,560 (20,00) | 0,7207 (21,35) | 0,5002 (22,45) |
| Linolenic | 0,0706 (3,10) | 0,0828 (3,3) | 0,0742 (2,65) | 0,0899 (2,8) | 0,0755 (3,12) |
| General content | 2,5 | 2,51 | 2,80 | 3,15 | 3,21 |

**Determination of the content of humic acids in purified mumiyo**

About 10 g (accurately weighed) of the drug is placed in a round-bottom flask with a capacity of 500 ml, 400 ml of 1% sodium hydroxide solution is added and heated in a water bath for 2 hours with frequent stirring and left for 24 hours at room temperature. From the settled alkaline solution with a pipette, take 100 ml into a flat-bottomed flask with a capacity of 300 ml and add 20 ml of 18% hydrochloric acid solution to precipitate humic acids. The resulting precipitate is filtered through an ashless dry, pre-weighed filter, washed with 10 ml of water. The filter with sediment is dried in a drying cabinet at a temperature of 100-1050C to constant weight. The difference determines the mass of the dry residue. The filter cake is transferred into a pre-calcined, weighed crucible and burned in a muffle furnace at a temperature of 600-6250C for 1-2 hours. Then the crucible is cooled in a desiccator, weighed and the ash mass is determined by the difference, and then the content of humic acids is calculated [9].

The content of humic acids in mumiyo is 7.24%. Table 3 shows the results of the determination.

**Table 3**

| Sample | Samples | | | | | Mean |
|---|---|---|---|---|---|---|
| | №1 | №2 | №3 | №4 | №5 | |
| Mumiyo peeled | 7,41 | 7,33 | 7,21 | 7,22 | 7,01 | 7,24 |

**Vitamin identification$D_3$.** Extraction of vitamin D_3 with hexane from the mumiyo dimethyl sulfoxide mixture and high-performance liquid chromatography; comparison of chromatograms of the test and standard samples. The chromatograph of the KNAUER company was used: column brand - Column: XB-C 18.5 µm; 4,6x250 mm. The stationary phase is a monomolecular layer of aminopropylsilane chemically bonded to porous silica gel particles. Column temperature is room temperature. The resolution factor between the peaks should not be less than 10. Mobile phase is a filtered and degassed mixture of n-hexane and isopropyl alcohol in a ratio of 99: 1; pump brand - HPLC PUMP K-501. Flow rate 1 ml / min; UV detector brand - UV Detektor K-2501. The absorption spectrum is added to 50 ml of dimethyl sulfoxide and 50 ml of n-hexane, then the mixture is shaken periodically in a water bath at 600C for 45 minutes. The resulting mixture was allowed to separate for 25 minutes and the n-hexane layer was removed with a pipette. To the remaining dimethyl sulfoxide layer, 80 ml of n-hexane are added, the mixture is stirred for 5 minutes at room temperature, and the n-hexane layer is removed with a pipette. This operation is repeated three more times, each time adding 25 ml of n-hexane. The combined hexane extracts are

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filtered through a filter paper, the filtrate is evaporated to dryness under vacuum at room temperature. The dry residue is dissolved in 10 ml of n-hexane and quantitatively transferred into a 15 ml pycnometer, the solution is brought to the mark with n-hexane. The resulting solution is filtered through a millipore filter, measured with a pipette 1 ml and transferred into a volumetric flask with a capacity of 25 ml, then the solution is brought to the mark with n-hexane. From this solution, 1 ml is measured again and the solution is prepared as described above. After the solution is chromatographed [10-11].

Thus, the conditions for chromatography have been found. The retention time of vitamin D_3 was 3 min. 10.5 sec. The quantitative content of vitamin D_3 in percentage (x) was determined by the following formula:

$\frac{S_1 \cdot m_0 \cdot 15}{S_0 \cdot m_1} \cdot 100$,where$S_0$ −peak area of the PCO of the vitamin$D_3$;$S_1$ −peak area of the tested mumiyo;$m_0$ −weight of RSO vitamin$D_3$; $m_1$ −weight of the test mumiyo in grams.The quantitative content of vitamin D_3 was 0.96%. This makes it possible to explain in a new way the acceleration of bone tissue healing after fractures and the prevention of impaired phosphorus-calcium metabolism under the influence of mumiyo. It is known that vitamin D_3 penetrates the cell membranes of the digestive tract and activates a gene that gives the command for the synthesis of a protein necessary for the transfer of calcium ions across the cell membrane; if the vitamin is absent, then calcium, despite its amount, cannot penetrate through the intestinal wall.

**Acute toxicity study.**The acute toxicity of the preparations was studied on 24 white mice weighing 19-21 g of mixed sex. The animals were divided into groups of 6 heads and once intragastrically injected with an aqueous suspension of drugs "Mumiyo asil ACP" capsules manufactured by Limited Liability Company (LLC) "PHARM ENGINEERING" and "Mumiyo Asil" manufactured by JV LLC "REMEDY GROUP", Uzbekistan in doses of 6000 mg / kg and 10000 mg / kg, which is 0.3–0.5 ml of suspension, respectively.

The animals were kept under continuous observation during the first hour, then under hourly observation during the first day of the experiment and once a day in the next 13 days of the experiment. As indicators of the functional state of animals, the general condition of mice and their behavior, the intensity and nature of motor activity, the presence of seizures, coordination of movements, reaction to external stimuli and tone of skeletal muscles, the frequency and depth of respiratory movements, the color of mucous membranes and the size of the pupil, appetite, were taken into account. body weight, quantity and consistency of fecal matter. During the experiment, the clinical condition of the animals was monitored: the presence / absence of signs of poisoning, the time of their appearance, the death of mice.

All experimental animals were kept in standard conditions, on a common diet with free access to water and food [13].

After the completion of the experiment, the average lethal doses (LD50) were determined [12].

**Results:**The experiments showed that after a single intragastric administration of the compared drugs at doses of 6000 mg / kg and 10000 mg / kg, no visible changes were observed in the behavior and functional state of the animals. All mice are active, respond to external stimuli, food and water consumption was normal. There are no pathological changes in the condition of the coat and skin and no signs of intoxication were observed. The consistency and amount of feces were unchanged. In this group, until the end of the experiment, no deaths among the animals were noted.

LD50 of the compared drugs was more than 10,000 mg / kg.

The experimental results are shown in Table 4.

**Table4**

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**Determination of acute toxicity (LD50) of the preparations "Mumiyo asil ACP" produced by LLC "PHARM ENGINEERING" and "Mumiyo asil" produced by JV LLC "REMEDY GROUP", Uzbekistan**

| № alive | "Mumiyo asil ACR", manufactured by LLC "PHARM ENGINEERING" Uzbekistan | | | | | "Mumiyo asil" produced by JV "REMEDY GROUP" LLC, Uzbekistan | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | the weight, r | dose, mg / kg | dose, ml | way introduction | fatal outcome | the weight, r | dose mg / kg | dose ml | way introduction | fatal outcome |
| 1 | 20 | 6000 | 0,30 | в/ж | Not | 21 | 6000 | 0,32 | in / f | Нет |
| 2 | 1920 | | 0,28 | | Not | 20 | | 0,30 | | Нет |
| 3 | 21 | | 0,30 | | Not | 19 | | 0,28 | | Нет |
| 4 | 1921 | | 0,32 | | Not | 20 | | 0,30 | | Нет |
| 5 | | | 0,28 | | Not | 20 | | 0,30 | | Нет |
| 6 | | | 0,32 | | Not | 21 | | 0,32 | | Нет |
| 1 | 19 | 10000 | 0,48 | в/ж | Not | 20 | 10000 | 0,50 | in / f | Нет |
| 2 | 19 | | 0,48 | | Not | 19 | | 0,48 | | Нет |
| 3 | 20 | | 0,50 | | Not | 20 | | 0,50 | | Нет |
| 4 | 20 | | 0,50 | | Not | 19 | | 0,48 | | Нет |
| 5 | 1920 | | 0,48 | | Not | 20 | | 0,50 | | Нет |
| 6 | | | 0,50 | | Not | 20 | | 0,50 | | Нет |
| $LD_{50}$ | >10000mg / kg | | | | | >10000mg / kg | | | | |

**Study of specific activity.**

The wound healing effect of the drug "Mumiyo asil ACR", produced by LLC "PHARM ENGINEERING" and "Mumiyo asil" by JV LLC "REMEDY GROUP", Uzbekistan was studied on 18 white rats weighing 180-210 g of both sexes. Reproduction of the model of planar skin wounds of the dorsal region in rats was carried out under general anesthesia using urethane at a dose of 1 g / kg. To inflict flat wounds, the hair and undercoat in the dorsal region were cut off and a skin flap of 400 mm2 was excised (removing also the subcutaneous fat). Skin defects were left open throughout the observation period. Compared drugs were administered within 25 days from the beginning of the experiment at a dose of 200 mg / kg.

The animals were divided into three groups of 6 animals each:

Group 1 - control;

Group 2 - experimental - intravenous drug "Mumiyo asil ACP", produced by LLC "PHARM ENGINEERING" at a dose of 200 mg / kg;

Group 3 - experimental - intravenous drug "Mumiyo asil" produced by JV LLC "REMEDY GROUP", Uzbekistan at a dose of 200 mg / kg.

After administration of drugs, the animals were placed in individual cages. The dynamics of wound healing was assessed by the change in its area using the formula: $S = A * B * \pi / 4$ (mm2), where A is the width, B is the length of the wound in mm. Measurements were carried out with an electronic caliper once every two days from the beginning of the experiment. The percentage of wound healing was calculated by the formula:

C = (Sbegin-S1 / Sbegin) * 100%,

where, S initial is the initial area of the wound, S1 is the area of the wound for the current day of measurement.

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After determining the area of wounds in experimental animals in each series, we calculated the average area (M ± m), the percentage of reduction in the area of wounds from the initial size (ie, the percentage of wound healing) [13].

The results obtained showed that in the control group of animals the area of the ulcerous surface one day after the operation decreased relative to the initial values and the percentage of healing was 5.5 ± 2.3%. Objectively, an increase in the inflammatory reaction was noted - swelling, hyperemia, infiltration of the surrounding tissues. On the 5-7th day, the area of the wound decreased relative to the initial proportion of healing was 22.6 ± 1.9%. On the 11-13th day of the experiment, a more pronounced effect of reducing the area of wounds was noted. At the same time, clinically, a decrease in edema, hyperemia, and infiltration of surrounding tissues was observed only on the 13th day. An insignificant white fibrinous plaque persisted. By the 15th day, at the site of the traumatic injury, bright pink, slightly protruding above the level of the surrounding tissue, slightly bleeding granulations were formed. There was a slight hyperemia of the surrounding tissues. By day 21, the percentage of healing was 78.8 ± 1.5% in relation to the initial value.

In the experimental group of animals, already from the moment of the beginning of the experimental therapy with the drug "Mumiyo asil ACP", produced by LLC "PHARM ENGINEERING", the inflammatory phenomena were less pronounced. Within 5, 7, 9 days, the proportion of wound healing was 32.2 ± 2.6%, respectively; 36.2 ± 1.6%; 46.8 ± 2.4% relative to the initial values and exceeded that in the control group by 17.6%, 13.6% and 13.7%, respectively. Clinically, on the 11th day of the experiment, the absence of hyperemia, edema was noted. The inflammatory response was less than that of the control group. On the 21st day of the experiment, the percentage of wound healing in the experimental group was 94.9 ± 0.3% and exceeded that in the control group by 16.1%.

In comparison with the group of animals that were injected with the drug "Mumiyo asil" manufactured by JV "REMEDY GROUP" LLC, Uzbekistan, it was found that the animals of the experimental group of the drug "Mumiyo asil ACP" produced by "PHARM ENGINEERING" LLC experienced a statistically significant acceleration of healing by On the e day of the experiment, it was 16.8% faster than in the comparison drug group. Complete healing of the wound defect in the group of the drug "Mumiyo asil ACP", manufactured by PHARM ENGINEERING LLC occurred on the 23rd day after the operation, and in the experimental group "Mumiyo asil" produced by JV REMEDY GROUP LLC, Uzbekistan on the 25th day. In animals of the control group that did not receive the drug, complete wound healing occurred by the 27th day after the operation.

The experimental results are shown in table 5.

**Table 5**
**Dynamics of changes in the area of wounds in rats during treatment**

| A day of experiment | The control | | "Mumiyo asil ACR" produced by "PHARM ENGINEERING" LLC Uzbekistan | | "Mumiyo asil" JV LLC "REMEDY GROUP", Uzbekistan | |
|---|---|---|---|---|---|---|
| | Area, wounds, mm2 | Share of healed,% | Area, wounds, mm2 | Share of healed,% | Area, wounds, mm2 | Share of healed,% |
| 1-e | 399,3±8,2 | - | 412,3±12,1 | - | 397,8±17,9 | - |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 3-и | 377,1±10,1 | 5,5±2,3 | 371,9±15,9 | 9,7±3,3 | 362,3±9,3 | 8,3±3,0 |
| 5-е | 341,2±14,9 | 14,6±3,1 | 278,6±8,9* | 32,2±2,6* | 333,8±7,6 | 15,4±3,2 |
| 7-е | 309,2±10,1 | 22,6±1,9 | 262,5±6,7* | 36,2±1,6* | 295,9±4,5 | 24,7±3,8 |
| 9-е | 266,4±7,6 | 33,1±2,5 | 218,2±7,5* | 46,8±2,4* | 269,9±6,1 | 31,4±3,4 |
| 11-е | 249,7±7,5 | 37,2±2,8 | 179,1±3,2* | 56,4±1,1* | 240,3±2,4 | 38,9±2,9 |
| 13-е | 215,1±8,4 | 45,8±3,1 | 131,4±7,3* | 68,0±1,8* | 217,9±3,6 | 44,4±3,4 |
| 15-е | 181,8±6,9 | 54,3±2,4 | 106,9±4,5* | 73,9±1,3* | 189,5±4,1 | 51,7±3,0 |
| 17-е | 147,9±4,7 | 62,8±1,9 | 65,8±3,8* | 84,0±0,9* | 133,7±7,9 | 65,7±3,3 |
| 19-е | 128,2±3,2 | 67,8±1,2 | 31,1±2,1* | 92,4±0,6* | 69,7±6,9* | 82,5±1,6* |
| 21-е | 84,5±5,3 | 78,8±1,5 | 20,8±1,6* | 94,9±0,3* | 31,5±2,2* | 91,9±0,7* |
| 23-е | 47,9±3,2 | 62,6±2,4 | 8,2±1,2 | 98,0±0,3* | 26,7±2,0* | 93,2±0,6* |
| 25-е | 25,4±1,9 | 69,5±2,3 | - | - | 9,2±0,5* | 97,6±0,2* |

Note: * - statistically significant in relation to the control group at P <0.05.

**Conclusions**:

1. A new method of purification of the Central Asian mumiyo, which was subjected to physical and chemical analyzes, was developed.
2. The chemical composition of mumiyo is characterized by the content of organic (gross formula $C_6H_6O_8$) and inorganic parts$CaSi(K,Na)_5C_{25}H_5O_{26}$
3. The inorganic part of mumiyo contains chemical elements that are both in a free state and in the form of oxides and salts and makes up about 30% of the total mass of mumiyo. Moreover, the share of potassium, calcium and magnesium accounts for more than 20% of the total mass of mumiyo.
4. To identify the organic part of mumiyo, a method based on distillation with water vapor was developed, as a result of which the sum of two groups of substances was obtained: hippuric acid and steroids.
5. Determination of the authenticity and good quality of the purified mumiyo was carried out by the data of IR spectra and color reactions.
6. A method for standardizing mumiyo by determining the amino acid composition has been developed; According to the data obtained, the analyzed sample contains 14 amino acids, of which 5 are irreplaceable; the absence of sulfur-containing (cysteine, cystine and methionine) amino acids is noted. The total content of glycine and glutamic acid exceeds 40% of the sum of amino acids. The total amino acid content in mumiyo is 3.2-4.5%
7. Mumiyo contains 12 fatty acids, the total content is 2.5-3.5%.
8. It has been established that the composition of mumiyo contains 7.24% of humic acids, as well as 96% of vitamin $D_3$
9. The obtained data show that the drug "Mumiyo asil ACP" - capsules of 150 mg, produced by PHARM ENGINEERING LLC in comparison with the drug "Mumiyo asil" - capsules of 150 mg, produced by JV "REMEDY GROUP" LLC, Uzbekistan in terms of acute toxicity is biologically equivalent.
10. Study of the wound healing effect of the drug "Mumiyo asil ACP" - capsules of 150 mg, manufactured by PHARM ENGINEERING LLC in comparison with the drug "Mumiyo asil" - capsules of 150 mg by JV "REMEDY GROUP" LLC, Uzbekistan showed that the drugs have wound healing action.

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